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Citation for published version:

Donaldson, K, Brown, GM, Brown, DM, Slight, J & Li, XY 1992, 'Epithelial and extracellular matrix injury in quartz-inflamed lung: role of the alveolar macrophage', *Environmental Health Perspectives*, vol. 97, pp. 221-4. <<http://www.jstor.org/stable/3431357>>

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Environmental Health Perspectives

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Epithelial and Extracellular Matrix Injury in Quartz-Inflamed Lung: Role of the Alveolar Macrophage

by Kenneth Donaldson,¹ Geraldine M. Brown,¹
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The bronchoalveolar leukocytes from quartz-inflamed lung were separated into macrophage-enriched and neutrophil-enriched populations on density gradients. Neutrophil-enriched populations showed the greatest activity in causing injury to epithelial cells and fibronectin *in vitro*. Inflammatory macrophage-enriched populations from quartz-exposed lung had the ability to cause fibronectin degradation but could not cause detachment injury to epithelial cells over and above that caused by control alveolar macrophages. Fibronectin damage *in vivo* could be an important factor in disordering the connective tissue scaffold of the lung, thereby favoring fibrosis. *In vitro* quartz stimulated more production of cytokines by alveolar macrophages than the inert particulate titanium dioxide. Cytokines could be important in upregulating adhesion molecules in the membranes of lung cells *in vivo*; this process could aid leukocyte/lung cell contact, allowing epithelial injury to be expressed, and could also be a factor leading to pathological change.

Introduction

Leukocytes perform an important defensive role in the lung, where they act to keep the alveolar epithelial surface free from particles. There is, however, evidence to suggest that leukocytes can also be harmful to the lung if they are recruited there in large numbers and become activated, as is found in workers in industries where the dust causes pneumoconiosis (1). Experimental inhalation of pneumoconiotic dusts such as quartz, asbestos, and coal mine dust causes macrophage and neutrophil recruitment (2,3). These leukocytes then have the potential to release a range of toxic products, including proteases, which we have demonstrated are able to injure epithelial cells (4) and extracellular matrix components (5) *in vitro*. In addition, leukocytes may also release fibroblast growth factors such as interleukin-1 (IL-1), which could contribute to fibrosis. In the presence of such growth factors, proteolytic injury to the connective tissue matrix of the lung after quartz exposure may lead to abnormal repair and fibrosis if the connective tissue scaffold is disordered. Epithelial injury can lead to disruption of the normal balance between the interstitium and the alveolar space. In addition, by stimulating proliferation of type II epithelial cells, quartz exposure can lead to the pathological lining of the alveoli with cells inappropriate to the gas transfer function of the alveoli and also accumulation of type II cell product.

In our own rat model of silicosis, both fibrosis and type II cell proliferation are evident (6) and, as described above, leukocytic

protease has the potential to play a major role. It is clear from our studies that neutrophils are a major source of the connective tissue protease (5), but alveolar macrophages also provide a potential source of harmful protease and are present generally in greater numbers than neutrophils. We therefore set out to determine whether macrophages from quartz-inflamed lung were able to injure epithelial cells and break down fibronectin *in vitro*. We also assessed the effect of different dusts on the ability of macrophages to release tumor necrosis factor (TNF) and interleukins 1 and 2 (IL-1 and IL-2).

Materials and Methods

Leukocyte Populations

Leukocyte populations were obtained from control PVG rats (<98% macrophages) or rats exposed to 1 mg of quartz by instillation 5 days previously (50% macrophages and 50% polymorphonuclear neutrophils [PMN]). Cells were collected by bronchoalveolar lavage as previously described (2). Cell populations were either kept whole or separated into macrophage and neutrophil-rich fractions by density gradient centrifugation through Sepacell medium according to the manufacturer's instructions (Septra-tech Corporation, Oklahoma City, OK).

Epithelial Injury

Epithelial injury was assessed by radiolabeling cells of the alveolar epithelial cell line A549 with ⁵¹Cr and incubating them with effector leukocytes for 6 hr at an effector: target ratio of 5:1. At the end of this time, two types of injury were assessed: lytic injury and detachment injury (4).

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Fibronectin Degradation

The ability of leukocytes to break down the extracellular matrix component fibronectin was assessed using fibronectin labeled with ^{125}I . ^{125}I -fibronectin was dried on to the base of microtiter plate wells and leukocytes were incubated on this matrix for 4 hr, and the release of ^{125}I -fibronectin breakdown products into the supernatant was measured (5).

Cytokine Production

Alveolar macrophages were exposed *in vitro* to various types of dust for 24 hr at 25 $\mu\text{g}/\text{mL}$ and the supernatants were collected. IL-1 in supernatants was measured as enhanced stimulation of suboptimal lectin-treated mouse thymocytes. TNF was measured using the L929 cell line.

Statistical Analysis

Data from repeat experiments were analyzed by analysis of variance to determine whether there were any treatment effects and to obtain a measure of estimated standard error. Differences between treatments were tested for statistical significance using this estimated error in a *t*-test.

Results

Epithelial Injury

As shown in Figure 1, the separated, macrophage-enriched populations from quartz-inflamed lung were able to cause detachment of epithelial cells. However, when individual experiments were assessed and detachment was related to the proportion of contaminating neutrophils (Fig. 2), it was clear that the detachment was attributable to the neutrophils. Results were obtained from four separate experiments using pooled cells from one to three rats in each experiment.

Fibronectin Degradation

As shown in Figure 3, the inflammatory macrophage-enriched population was capable of degrading large quantities of the extracellular matrix component fibronectin compared to control macrophages. However, in this case the increased proteolytic injury could not be explained by the 4% of contaminating PMN. This level of PMN would be anticipated to increase the proteolytic activity of the macrophage population by 12%, whereas the actual increase over the control macrophages was 45%. Results represent means and standard deviations from five separate experiments, with cells from one rat used in each experiment.

Cytokine Production

We have demonstrated, as shown in Figure 4, that quartz caused more release of cytokine from alveolar macrophages *in vitro* than titanium dioxide. On dilution, the supernatants showed a sigmoid curve of activity, as is normally seen with crude supernatant in a bioassay of this sort. It is presumed that the differential effect of dilution on the response is due to conflicting activities present in the supernatant, which dilute out at different rates to the TNF activity.

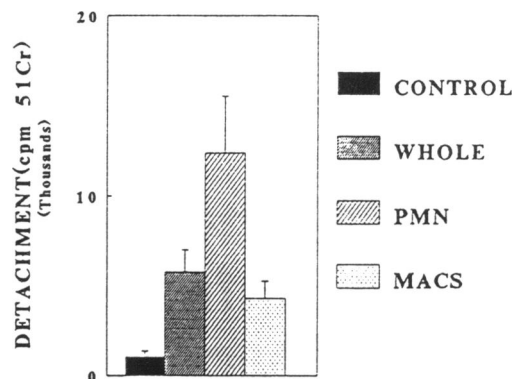


FIGURE 1. Detachment injury caused to epithelial cells by control bronchoalveolar leukocytes and cells from quartz-exposed lung. Whole, unseparated; PMN, PMN-enriched population; MACS, macrophage-enriched population. Significantly more detachment caused by all quartz populations compared to control macrophages ($p < 0.01$). Significantly more detachment caused by PMN than MACS or whole ($p < 0.05$).

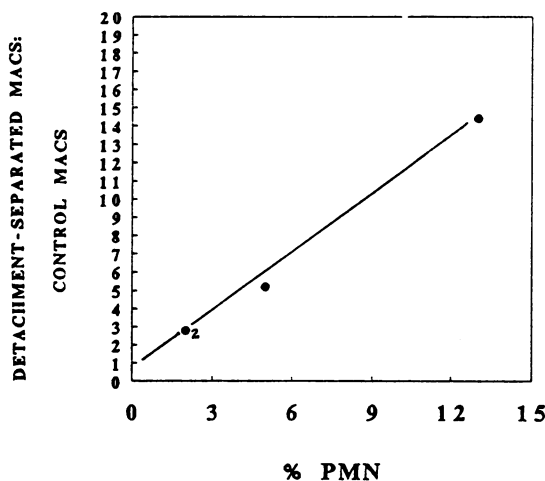


FIGURE 2. Detachment injury, expressed as detachment caused by macrophage-enriched populations divided by detachment caused by control macrophage populations, versus percentage of contaminating PMN. The line, fitted by eye, passes through 1, suggesting that inflammatory macrophages cause as much detachment as control macrophages when no PMN are present. Data from four separate experiments. The "2" represents two data points that were identical.

Discussion

We and others have demonstrated the ability of PMN to cause injury to epithelial cells and extracellular matrix components (4). Macrophages are present in normal lung but are present in increased numbers and in an activated state in dust-inflamed lung (2) and so could also contribute to injury. We have shown here that macrophages from inflamed lung are unlikely to be able to cause epithelial damage, at least in the rat model of silicosis that we used here. In contrast, the inflammatory macrophages have the ability to break down fibronectin in increased amounts compared to control macrophages.

In the case of PMN, we have previously associated the ability to break down fibronectin with the ability to cause epithelial injury, both of which are protease-mediated events (4). We report

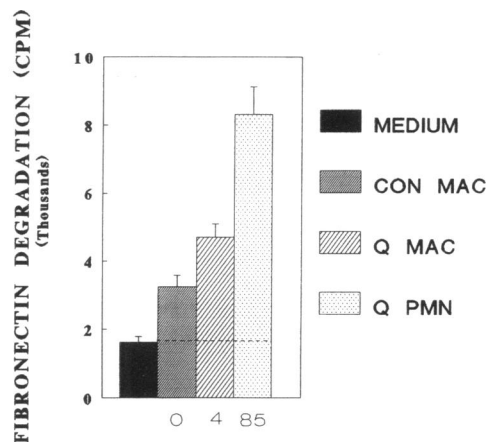


FIGURE 3. Fibronectin degradation caused by control alveolar macrophages (CON), macrophage-enriched (Q MAC), or PMN-enriched (Q PMN) populations from quartz-inflamed lung. Spontaneous release of 125 I-fibronectin counts are shown as the bar marked "medium." Numbers under the bars indicate the mean percentages of PMN. Results are the means \pm SDs of five separate experiments, with two to three rats used in each experiment. Significantly more fibronectin degradation with all populations compared to medium alone ($p < 0.01$) and with Q PMN compared to the other conditions.

here that the two functions are not necessarily associated and that macrophages have the ability to break down fibronectin in the absence of the potential to cause detachment injury to epithelial cells *in vitro*.

It seems likely that close-range interaction, possibly comprising membrane-membrane contact with target cells or adherence to extracellular matrix components, is a necessary prerequisite for inflammatory leukocytes to cause injury. Cell-to-cell attachment involves the integrin class of molecules and associated adhesion molecules in the cell membrane (7). Clearly, both macrophages and neutrophils possess the receptors that allow close interaction between these cells and fibronectin. However, only neutrophils may possess the receptors that allow close interaction with epithelial cells *in vitro*.

We have found that the inflammatory dust quartz, which was used as a model injuring agent in the present study, is able to directly stimulate the release of the cytokines IL-1 and TNF from alveolar macrophages in excess of that produced when the macrophages are treated with the relatively harmless dust titanium dioxide (2). Cell adhesion molecules can be upregulated by exposure to cytokines (8). Thus, in the lung itself, when the epithelial cells are exposed to the cytokine for longer than the 5 hr used in the assay here, this time may allow upregulation of adhesion molecules on the epithelial cells, allowing macrophage-mediated short-range epithelial injury to occur *in vivo*.

We intend to expose epithelial cells to cytokine *in vitro* and then assess the ability of the macrophages to cause injury. This may allow the epithelial cells to upregulate their adhesion molecules, which could promote close contact between the effectors and the targets, possibly allowing injury to be expressed. The fact that PMN can cause the injury may be related to expression of the appropriate adhesion molecules or their much increased production of protease, which may be sufficient to cause injury without the necessity for direct contact. We have, however, demonstrated

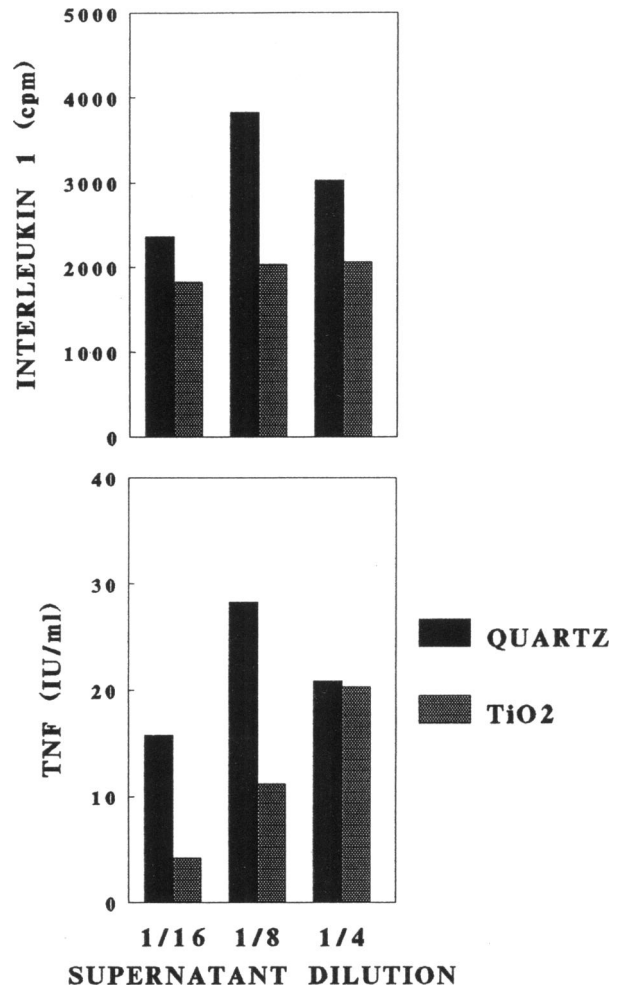


FIGURE 4. Production of interleukin-1 (IL-1) and tumor necrosis factor (TNF) by alveolar macrophages exposed to quartz and titanium dioxide (TiO₂) *in vitro*. Results are expressed as thymocyte proliferation in counts per minute (IL-1) or international units per milliliter (TNF). Results are shown as the means of three to five experiments with significant differences between TiO₂ and quartz at 1/8 and 1/16 dilutions ($p < 0.05$). Pooled cells from one to three rats were used in each experiment.

the production of high levels of IL-1 by neutrophils from inflamed lung (9), and this may also play a role.

This research was funded by the Colt Foundation.

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